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Clinical utility of endocrine markers predicting myocardial siderosis in transfusion dependent thalassemia major

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Abstract

Background: Endocrinopathy due to iron overload is the most common morbidity whereas myocardial siderosis causing toxic cardiomyopathy is the leading cause of mortality among patients with transfusion dependent thalassemia major (TDTM). If detected early, this can be treated with aggressive chelation. T2* cardiac magnetic resonance imaging (CMR) guided chelation protocols are now the gold standard but have limited availability in low and middle-income countries. We hypothesized that markers of endocrine dysfunction would correlate with T2* CMR and can be used to predict the severity of myocardial siderosis and guide chelation therapy.

Methodology: We undertook a multicenter retrospective study of 280 patients with TDTM to assess the prevalence of endocrinopathies and the predictive value of a number of individual and composite markers of endocrinopathy with T2* CMR.

Results: The prevalence of hypogonadism, stunting, hypoparathyroidism, and hypothyroidism was 82%, 69%, 40%, and 30%, respectively. The sensitivity of hypogonadism and stunting predicting severe myocardial siderosis was 90% and 80%, respectively.

Conclusion: We conclude that clinical markers of endocrine dysfunction, especially hypogonadism (positive likelihood ratio [LR+] = 1.4, 95% confidence interval [CI] = 1.0–1.9; positive predictive value [PPV] = 77%, 95% CI = 70–82; negative predictive value [NPV] = 57%, 95% CI = 34–77] and stunting (LR+ = 1.3, 95% CI = 1.1–1.6; PPV = 64%, 95% CI = 60–69; NPV = 55%, 95% CI = 45–64) in TDTM can predict severe myocardial siderosis and can potentially guide chelation therapy, especially where access to T2* CMR is limited.

KEYWORDS

β-thalassemia major, endocrinology, thalassemia

1 | INTRODUCTION

Repeated blood transfusions in transfusion dependent thalassemia major (TDTM) can lead to significant organ siderosis unless controlled with chelation.[1,2] Endocrine complications due to organ siderosis are the leading cause of morbidity,[3,4] whereas myocardial iron deposition is most significantly associated with mortality.[5–7] Given the unreliability of serum ferritin and liver iron deposition as a marker of myocardial iron overload,[8,9] T2* cardiac magnetic resonance imaging (CMR) guided chelation protocols are now the gold standard[6,7] but have limited availability in low and middle-income countries (LMIC) such as Pakistan.[10] Aggressive chelation can potentially reverse endocrinopathies[11]; however, these data are lacking for our population.

The preliminary T2* CMR based study from Pakistan reported that 47% of our TDTM population had severe myocardial siderosis.[12] Significant endocrinopathies, especially hypogonadism and hypoparathyroidism,[13,14] have also been reported. The majority of these patients are suboptimally chelated.[15] Due to lack of governmental support or

ABBREVIATIONS: AKUH, Aga Khan University Hospital; CI, confidence interval; FSH, follicle stimulating hormone; LH, luteinizing hormone; LMIC, low and middle-income countries; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; CMR, cardiac magnetic resonance imaging; TDTM, transfusion dependent thalassemia major

insurance, our major challenge is the lack of laboratory evaluation of endocrinopathies as it becomes difficult to cover the cost since most of the patients pay out of their pocket.

Hence, in order to decrease morbidity and mortality, we aimed to find a reliable predictor of myocardial iron overload where there was limited availability of T2* CMR. We hypothesize that the association between the most common morbidity, endocrine dysfunction,[3,4] and the degree of myocardial siderosis can provide a predictive tool to potentially guide chelation therapy in a cost-effective manner with decreased reliance on expensive laboratory tests. We understand that this does not replace the need for CMR in the long-term follow-up of patients, and thus our method of estimating myocardial siderosis mainly focuses on screening high-risk patients on initial presentation.

2 | METHODS

2.1 | Study population and design

A multisite registry—aimed at improving management of iron overload—for patients who have undergone T2* CMR at one of our four centers (Table 2) has included 305 patients since its inception in 2014. A total of 280 patients from the registry with endocrine assessment, added till December 2016, were selected for the purpose of this study. All patients had T2* CMR done at the Aga Khan University Hospital (AKUH), Karachi, given that this is the only center in Pakistan with a T2* CMR facility. Patients with thalassemia intermedia, non-transfusion-dependent thalassemia, inadequate endocrine work-up, or post bone marrow transplant were excluded.

2.2 | Diagnostic criteria

Algorithm and reference ranges of biochemical markers described in Table 1 were used to establish a diagnosis of endocrinopathy among these patients with TDTM.

2.3 | Iron overload

Detailed T2* CMR was performed at AKUH as previously described.[16] Briefly, short-axis midventricular slices were acquired in single breath holds at 10 echo times between 1.9 and 20.26 msec with a time interval of 2.03 msec between each slice. We used a combination of automated truncation[17] and a best fit-to-curve model to report our T2* values.[18] Cardiac iron load was considered normal if the T2* CMR value was >20 msec, mild to moderate if T2* CMR = 10–20 msec, and severe myocardial siderosis if the T2* CMR value was <10 msec.[19] Iron overload based on serum ferritin levels > 2500 μg/l, an average of minimum one and maximum five recent values, whichever was available,[12,20] was considered severe,[3] and measured via standard enzyme immunoassay at the different centers (Ferritin Calibrator; Hoora Pharma, and Immulite 2000 Processor, Siemens Healthcare Global, Erlangen, Germany).

TABLE 1 Diagnostic criteria for evaluation of endocrinopathies among patients with TDTM; values inside brackets indicate the normal/cut-off values for each endocrine laboratory test

Endocrinopathy		Diagnostic criteria
Hypogonadism (females > 13 years old and males > 14 years old)	Hypogonadotropic	LH (male: 1.2–7.8 mIU/ml, female: 3.5–56.6 mIU/ml): normal or low FSH (male: 1.4–15.4 mIU/ml, female: 1.4–9.2 mIU/ml): normal or low Testosterone (male: 249–836 ng/dl): low Estradiol (female: 9.5–24.2 pg/ml): low
	Hypergonadotropic	LH: high FSH: high Testosterone (males): low Estradiol (females): low
Stunting		Height z-score according to age < 2 SD
Hypoparathyroidism	PTH (16–87 g/ml): low or normal Calcium (8.6–10.2 mg/dl): low Phosphorus (≤15 years old: 3.2–5.8 mg/dl, >15 years old: 2.4–4.4 mg/dl): high[a]	
Hypothyroidism	Primary	FT4 (0.86–1.76 ng/dl): low TSH (low: <5 μIU/ml, lower tier: 5–10 μIU/ml, upper tier: >10 μIU/ml): upper tier
	Subclinical A	FT4: normal TSH: lower tier
	Subclinical B	FT4: normal TSH: upper tier
	Secondary	FT4: low TSH: low or normal

[a]Further details of cut-off values and enzyme immunoassays are given in Supplementary Document S1.
FT4, free thyroxine; PTH, parathyroid hormone; TSH, thyroid stimulating hormone.

2.4 | Endocrine assessment

Data for the gonadal axis were available for 95 patients with TDTM; females (n = 52) and males (n = 43) ≥13 and 14 years old, respectively, had been selected. Tanner staging of external genitalia, breast development (for females), and pubic hair (for males and females), as established by Marshall and Tanner,[21,22] was done. Serum endocrine markers, that is, luteinizing hormone (LH), follicle stimulating hormone (FSH), total testosterone, and estradiol were measured.

Data for height and weight were available for 263 patients; height and weight were measured using standard, regularly calibrated weighing machines and stadiometers.

Data for the parathyroid axis (assessed using parathyroid hormone, calcium, and phosphorous) were available for 152 patients and those for the thyroid axis (using thyroid stimulating hormone and free thyroxine) were available for 149 patients.

Appropriate cut-off values respective to enzyme immunoassays were used for each endocrine marker (Supplementary Document S1)

2.5 | Statistical analysis

Data were analyzed using IBM SPSS Statistics v. 22.0. Continuous data were expressed as median with interquartile range (IQR). Categorical variables were expressed as frequencies and their percentages. Univariate logistic regression analysis was done to determine the strength of the association between individual and composite endocrine abnormalities and T2* CMR. For diagnostic evaluation of each endocrine abnormality, its sensitivity, specificity, and positive likelihood ratio at 95% confidence interval (CI) were calculated based on the severity of iron load measured via T2* CMR.

3 | RESULTS

3.1 | Patient characteristics

Of the 280 patients with TDTM with endocrine work-up, 158 (56.4%) were males; 147 (52.5%) of the patients with TDTM were primarily followed at AKUH, 49 (17.5%) at the Fatimid Foundation, 21 (7.5%) at Kashif Iqbal Thalassemia Care Center, and 21 (7.5%) at Afzaal Memorial Thalassemia Foundation. Median age of the cohort at the time of TDTM diagnosis was 6 months (IQR: 8 months) and at presentation for T2* CMR was 17 years (IQR: 7 years). Median duration for receiving blood transfusions was 13 years (IQR: 8 years) (Table 2).

TABLE 2 Basic characteristics of patients with TDTM presenting for T2* CMR

Patient characteristics		Frequency (%)
Centers	AKUH	147 (52.5%)
	Fatimid Foundation (FF)	49 (17.5%)
	Kashif Iqbal Thalassemia Care Center (KITCC)	21 (7.5%)
	Afzaal Memorial Thalassemia Foundation (AMTF)	63 (22.5%)
Gender (male)		158 (56.4%)
Age in years (median with IQR)		17 (7.0)
Height, cm (median with IQR)		142 (22.3)
Weight, kg (median with IQR)		36 (16.9)
Body mass index, kg/m^2 (median with IQR)		17 (4.0)
Age (in months) at diagnosis (median with IQR)		6.0 (8.0)
Years on transfusion (median with IQR)		13 (8.0)
Frequency of transfusion, per month (median with IQR)		2 (1)
Number of packed red blood cell units (PRBC) transfused at each visit (1 PRBC is approx. 300 ml) (median with IQR)		1 (1)
Active hepatitis status	HBV	3 (2.0%)
	HCV	64 (38.3%)
HIV status positive		5 (3.9%)

3.2 | Endocrine complications

The prevalence of endocrine complications is reported in Table 3. Hypogonadism had the highest prevalence (n = 78, 82.1%), with its hypogonadotropic subtype being more common (n = 72, 92.3%), followed by stunting (n = 182, 69.2%), hypoparathyroidism (n = 61, 40.1%), and hypothyroidism (n = 45, 30.2%).

3.3 | Myocardial siderosis

Forty-eight (17.1%) patients had mild to moderate and 140 (57.9%) patients had severe iron overload based on T2* CMR. No significant correlation was seen between the average ferritin value and severe myocardial siderosis depicted by T2* CMR < 10 msec (r = −0.071, *P*-value = 0.446).

3.4 | Association between endocrine complications and T2* CMR

Median time difference between evaluation at endocrinology labs and T2* CMR was 1.3 years (IQR: 5.0); T2* CMR was done after evaluation at endocrine labs for some patients due to delay in getting an appointment or, initially, due to unavailability of T2* CMR. Hypogonadism, stunting, hypoparathyroidism, and hypothyroidism, all predicted myocardial siderosis in the univariate model. Hypogonadism had the highest odds of predicting severe iron overload (OR = 4.38, *P* = 0.017, 95% CI = 1.30–14.78), followed by stunting, hypoparathyroidism, and hypothyroidism, respectively (Table 4). T2* CMR showed a weak negative correlation with age (Supplementary Figure 1); however, it was not statistically significant.

3.5 | Test validation

We observed that hypogonadism (sensitivity 90%, CI 80–100) and stunting (sensitivity 80%, CI 70–80) were the two most sensitive markers of severe iron overload (T2* CMR < 10 msec). A normal thyroid or parathyroid axis predicted the absence of severe iron overload (specificity of 80% [CI 70–90] and 70% [CI 60–80], respectively). Additionally, absence of a combination of at least any three complications had 90% specificity in ruling out severe iron overload, as shown in Table 5. Positive predictive value of hypogonadism was 77% (CI 70–82), stunting 64% (CI 60–69), hypoparathyroidism 72% (CI 61–81), and hypothyroidism 73% (CI 59–84). Negative predictive value of hypogonadism was 57% (CI 34–77), stunting 55% (CI 45–64), hypoparathyroidism 46% (CI 39–53), and hypothyroidism 49% (CI 43–55).

4 | DISCUSSION

In an LMIC with a high burden of TDTM,[23] poor chelation, and very limited availability of T2* CMR, we found a higher prevalence of endocrinopathies as compared to other countries. The presence of

TABLE 3 Prevalence of endocrine complications and their subtypes among patients with TDTM presenting for T2* CMR

Endocrine complication	n (%)	Subtype of endocrine complication	n (%)
Hypogonadism	78/95 (82%)	Hypogonadotropic	72 (92%)
		Hypergonadotropic	2 (2.6%)
		Unknown	4 (5.1%)
Stunting	182/263 (69.2%)	–	–
Hypoparathyroidism	61/152 (40.1%)	–	–
Hypothyroidism	45/149 (30.2%)	Subclinical A	6 (13.3%)
		Subclinical B	3 (6.7%)
		Primary	15 (33.3%)
		Secondary	19 (42.2%)
		Unknown	2 (4.4%)

TABLE 4 Univariate logistic regression analysis showing the strength of association between endocrine complications and iron overload based on T2* CMR among patients with TDTM

Endocrine complication	OR	*P*-value	95% CI
Hypogonadism	4.38	0.017	1.30–14.78
Stunting	2.18	0.007	1.24–3.85
Hypoparathyroidism	2.18	0.048	1.01–4.71
Hypothyroidism	2.60	0.028	1.10–6.00

endocrinopathies, specifically hypogonadism or stunting, had a statistically significant high sensitivity for predicting myocardial siderosis.

We found a higher prevalence of endocrinopathies in our population—hypogonadism 82% (global prevalence = 40.5%), stunting 69% (global prevalence = 30.8%), hypoparathyroidism 40% (global prevalence = 6.9%), and hypothyroidism (global prevalence = 3.2%).[3] The prevalence calculated in our study was comparable to studies done in Pakistan, which reported 90–100% prevalence of hypogonadism and 40% prevalence of parathyroid dysfunction.[14,24] The high prevalence of endocrinopathies in our population is likely due to the higher prevalence of severe iron overload, as reflected by T2* CMR. This is demonstrated by the fact that 60% of our patients had a T2* CMR value of <10 msec, findings that are in line with what we reported in our preliminary study[12]; this degree of iron overload is much higher compared to international and regional prevalence, which ranges from 25% to 44%.[25–27] This iron overload is not only a reflection of the inadequately managed chelation therapy in our setting[26] but also of the delayed age of presentation of our patients (17 years [IQR: 7 years]); increasing age has been shown to be a risk factor for developing endocrinopathies.[28]

Prior to T2* CMR, serum ferritin was used as a marker of siderosis to guide chelation therapy. Due to its poor correlation with T2* CMR and its variation during inflammation,[29–32] there is a need for an alternative marker to guide chelation. This especially holds true in populations such as ours where there is a high prevalence of blood transfusion transmitted infections.[12,33–35] Hence, serum ferritin has little to no value in the assessment of myocardial siderosis.

Currently, in resource-limited settings with severe myocardial iron overload, delayed age at presentation of the patients, and unavailability of T2* CMR, the need for surrogate markers for assessing iron overload is necessary. It is especially true in our context as due to lack of governmental support or insurance, most of the patients are paying out of their pocket which results in a lack of laboratory tests or a lack of follow-up after laboratory tests as advised. Such markers may not replace the need for T2* CMR but may help prioritize the patients in need of aggressive chelation and urgent T2* CMR versus those who have the option of less aggressive chelation and electively opting for T2* CMR.[10] In our study, we showed that dysfunction of the endocrine axis in patients with TDTM had a high association with severe myocardial iron overload, indirectly reflecting the need for diagnosing endocrinopathies in order to reduce mortality. This was supported by historical studies done at the Ferrara Centre[36] from 1980 to 2007 and that done by Jensen et al.[37] in 1997, which explained the presence of multiple endocrinopathies in patients with TDTM due to siderosis. Presence of hypogonadism and stunting showed a high sensitivity—90% and 80%, respectively—for predicting severe iron overload. This was comparable to a previous study which reported LH, FSH, and testosterone as surrogate markers and indicators of iron overload (T2* CMR < 20 msec) in the absence of T2* CMR.[10] The absence of endocrine dysfunction was also shown to be associated with better T2* CMR values and controlled myocardial iron overload.

Clinical examination via Tanner staging and anthropometry with height z-scores can be used to indicate the presence of hypogonadism and stunting, respectively;[3,38] they provide cheap and efficient alternatives to expensive biochemical laboratory testing in order to assess the aforementioned endocrinopathies. Such clinical exam based markers are highly beneficial for resource-limited settings and can serve as a surrogate for T2* CMR until it is feasible to get it done. This could potentially improve both endocrine-related morbidity and cardiac-related mortality in our patients, laying the foundation for management of iron overload before siderosis-related complications even develop.

4.1 | Strengths

This was a large multicenter study, providing more complete data on endocrine complications among patients with TDTM in Pakistan than reported previously. Moreover, the use of T2* CMR, the standard

TABLE 5 Sensitivity, specificity, and positive likelihood ratio of endocrine complications and their combinations to evaluate their diagnostic capability of ruling in severe iron overload based on T2* CMR among patients with thalassemia major

Endocrine complication	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio (95% CI)
Hypogonadism	46	14	6	8	0.9 (0.8–1.0)	0.4 (0.2–0.6)	1.4 (1.0–1.9)
Stunting	99	55	33	40	0.8 (0.7–0.8)	0.4 (0.3–0.5)	1.3 (1.1–1.6)
Hypoparathyroidism	36	14	39	33	0.5 (0.4–0.6)	0.7 (0.6–0.8)	1.6 (1.0–2.7)
Hypothyroidism	27	10	43	41	0.4 (0.3–0.5)	0.8 (0.7–0.9)	1.9 (1.1–3.7)
Hypoparathyroidism and hypothyroidism	13	3	47	34	0.2 (0.1–0.3)	0.9 (0.8–1.0)	2.7 (0.8–8.8)
Hypoparathyroidism and hypogonadism	23	4	22	13	0.5 (0.4–0.7)	0.8 (0.5–0.9)	2.2 (0.9–5.4)
Hypoparathyroidism and stunting	25	7	43	36	0.4 (0.3–0.5)	0.8 (0.7–0.9)	2.7 (1.1–4.8)
Hypothyroidism and hypogonadism	15	5	30	15	0.3 (0.2–0.5)	0.8 (0.5–0.9)	1.3 (0.6–3.2)
Hypothyroidism and stunting	17	5	48	41	0.3 (0.2–0.4)	0.9 (0.8–1.0)	2.4 (1.0–6.1)
Hypogonadism and stunting	32	7	16	14	0.7 (0.5–0.8)	0.7 (0.4–0.9)	2.0 (1.1–3.8)
Hypoparathyroidism, hypothyroidism, and hypogonadism	10	2	30	14	0.3 (0.1–0.4)	0.9 (0.6–1.0)	2.0 (0.5–8.1)
Hypoparathyroidism, hypothyroidism, and stunting	10	2	47	34	0.2 (0.1–0.3)	0.9 (0.8–1.0)	3.0 (0.7–12.8)
Hypoparathyroidism, hypogonadism, and stunting	17	2	26	15	0.4 (0.2–0.6)	0.9 (0.6–1.0)	3.4 (0.9–13.0)
Hypothyroidism, hypogonadism, and stunting	12	2	29	17	0.3 (0.2–0.5)	0.9 (0.7–1.0)	2.8 (0.7–11.2)
Hypoparathyroidism, hypothyroidism, hypogonadism, and stunting	9	1	27	14	0.3 (0.1–0.4)	0.9 (0.7–1.0)	3.8 (0.5–27.1)

TP, true positive; FP, false positive; FN, false negative; TN, true negative

of care for TDTM management, provided the most accurate picture of the iron overload in our patients, as opposed to previous studies from resource-limited settings which assessed iron overload via serum ferritin.

4.2 | Limitations

Our study is limited by the fact that it is a retrospective chart-based review involving multiple centers, and not all the patients underwent an endocrine work-up using the same uniform protocol, which led to some gaps in the hormonal assessment of patients. Since this is not a prospective study, there was variability among patients with regard to the time frame from initial recommendation of T2* CMR and endocrine clinical evaluation to endocrine laboratory tests; hence, we were only able to provide the median time difference between T2* CMR and evaluation at endocrine labs. In addition, the centers used different assays for the various biochemical markers, probably adding some variability to the values of the hormones measured. That being said, we found no major differences in the prevalence of various endocrinopathies among the centers.

As AKUH is the only center that performs T2* CMR in our country, we may have an overrepresentation of patients with severe iron overload. The fact that around half of our patients were from other centers reassures us though, that we have an adequate representative population of patients with TDTM from Pakistan. Moreover, since breath holding is needed for T2* CMR, we were only able to recruit patients who were aged 8 years or more. We are attempting to further proceed toward methods that do not rely on breath holding for measurement of organ iron overload.

Although this study provides an insight into the current situation of endocrine complications among patients with TDTM, we recommend that further work be done in order to assess the prevalence of adrenal dysfunction and glycemic abnormalities in our population along with exploring more alternative methods of iron overload measurement and relating them to chelation therapy in order to improve the quality of life of our patients with TDTM. However, due to lack of data in our population with regard to iron chelation potentially reversing endocrinopathies, we do acknowledge that the endocrinopathy maybe not be irreversible. Therefore, our findings may have the greatest clinical utility at initial presentation.

5 | CONCLUSION

Endocrine complications remain one of the most frequent morbidities with devastating consequences that may be avoided if iron overload is identified and properly managed via chelation therapy. Our multicenter study reported a prevalence that was higher than the global prevalence of endocrine complications which strongly predicted severe myocardial iron overload measured via T2* CMR. Specifically, presence

of hypogonadism or stunting was reported to have a high sensitivity for suggesting severe myocardial iron overload. Hence, we recommend that hypogonadism via Tanner staging and stunting via anthropometry be assessed and an intervention be made without awaiting T2* CMR results; this allows for timely and cost effective management among patients with TDTM in an LMIC setting.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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